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(71) Applicant: EASTMAN KODAK COMPANY [US/US]; 343 State Street, Rochester, NY 14650 (US).

(72) Inventors: BELLY, Robert, Troconis ; 736 Blue Creek Drive, Webster, NY 14580 (US). MICHNO, Drake, Matthew ; 9 Green Pine Lane, Webster, NY 14580 (US). FLECKENSTEIN, Lee, Joseph ; 66 Winterset Drive, Rochester, NY 14625 (US). WASHBURN, William, N. ; 8686 Dunaway Drive, La Jolla, CA 92037 (US).

(74) Agent: EVERETT, John, R.; 343 State Street, Rochester, NY 14650 (US).

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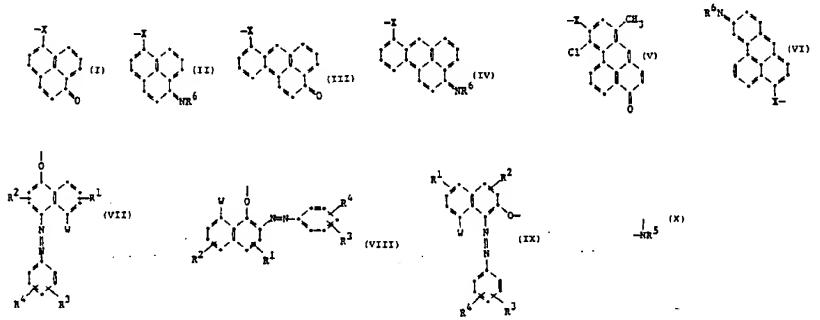

(57) Abstract

Compounds and reagents are disclosed for detecting oxidase positive organisms. The compounds have the structure: COUP-(LINK)$_n$-R, wherein COUP- represents a radical that couples with an oxidized primary amine and releases -LINK-R; -LINK- represents a divalent radical that undergoes intramolecular cyclization and release of -R upon release by COUP- ; n represents zero or one; -R represents a monovalent radical that forms a detectable species in the form a colorimetric dye or fluorescent compound upon release from -LINK-; wherein -R is selected from the group consisting of formulae (I), (II), (III), (IV), (V), (VI), (VII), (VIII) and (IX), wherein W represents hydrogen; halogen; hydroxy; substituted or unsubstituted carbonamido; sulfonamido; sulfonyl; ureido or amino; R^1 and R^2 each independently represent hydrogen, halogen, alkyl, alkoxy, carboxy, sulfo, cyano, nitro, carboxylic acid ester, carbonyl, sulfonyl, carbonamido, sulfonamido, alkysulfonyl, arysulfonyl; and R^3 and R^4 each independently represent halogen, nitro, sulfonamido, sulfonyl, carbonamido, carbonyl, cyano, alkylsulfonyl, arysulfonyl; R^6 represents H, CH_3 or C_2H_5; X represents -O-, -S- or formula (X), and R^5 represents H, alkyl, cycloalkyl or aryl.

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NOVEL COMPOUNDS AND REAGENTS FOR OXIDASE TEST

The present invention relates to novel compounds and their use as reagents in methods and elements for detecting oxidase positive organisms.

Dye forming reactions are used in the oxidase test for detection of oxidase-positive organisms.

The oxidase test is based on the bacterial production of an intracellular oxidase enzyme. See Biochemical Tests for Identification of Medical Bacteria, p. 250, J. MacFaddin, Williams and Wilkins Co. Oxidase-positive organisms include Pseudomonadaceae, Moraxella, Nesseria, Aeromonas, Vibrionaceae and Pleisiomonas shigelloides.

Oxidase-positive organisms produce reduced cytochrome c oxidase. It is oxidized in the presence of oxygen. Oxidized cytochrome c oxidase oxidizes primary amines such as dimethyl-p-phenylenediamine which in turn reacts with α-napththol to form indophenol blue. The formation of indophenol blue signals the presence of an oxidase-positive organism.

The problem is that the reaction of oxidized dimethyl-p-phenylenediamine and α-napththol, however, provides a relatively insensitive test and the resulting dye is unstable, thus failing to indicate the presence of oxidase-positive organism in some cases.

The present invention provides an alternative test that can be used when the prior art test fails. The test uses a reagent comprising:

a) a hydrogen donating primary amine; and

b) a compound selected from those having the general formula

$$COUP\text{-}(LINK)_n\text{-}R$$

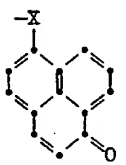
wherein

COUP- represents a radical that couples with an oxidized primary amine and releases -LINK-R;

-LINK- represents a divalent radical that undergoes intramolecular cyclization and release of -R upon release by COUP-;

n represents zero or one;

-R represents a monovalent radical that forms a detectable species in the form of a colorimetric dye or fluorescent compound upon release from -LINK-; wherein -R is selected from the group consisting of:



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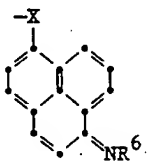

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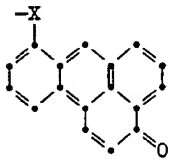

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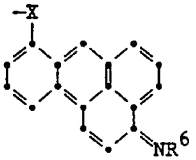

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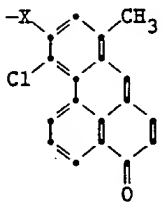

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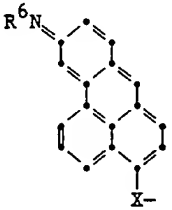

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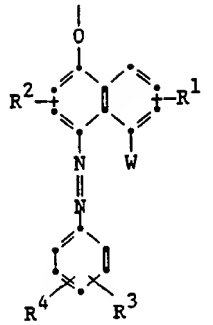

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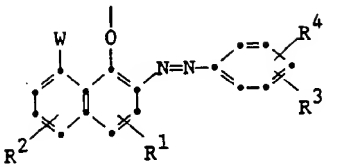

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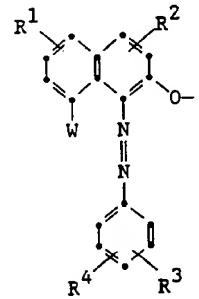


; wherein

W represents hydrogen, halogen such as chloro or bromo; hydroxy; or substituted or unsubstituted carbonamido such as acetamido or sulfonamido; sulfonyl, ureido or amino;

R^1 and R^2 each independently represent hydrogen, halogen, alkyl such as methyl, ethyl or propyl, alkoxy such as methoxy, t-butoxy, carboxy,

sulfo, cyano, nitro, carboxylic acid ester, carbonyl, sulfonyl, carbonamido, sulfonamido, alkylsulfonyl such as methanesulfonyl, arylsulfonyl such as benzenesulfonyl; and

R^3 and R^4 each independently represent halogen, nitro, sulfonamido, sulfonyl, carbonamido, carbonyl, cyano, alkylsulfonyl such as methane-sulfonyl, and arylsulfonyl such as benzenesulfonyl;

R^6 represents H, CH_3 or C_2H_5;

X represents -O-, -S- or $-\overset{|}{N}R^5$; and

R^5 represents H, alkyl such as methyl, ethyl or butyl, cycloalkyl such as cyclohexyl or aryl such as phenyl or napthyl.

The compounds COUP-(LINK)-R combine with hydrogen donating primary amines (referred to as amine hereinafter) to form reagents which, in the presence of oxidase positive organisms, release a reporter compound in the form of (1) colorimetric dyes having high extinctions and absorptions at wavelengths greater than 500 mm or (2) fluorescent dyes having absorptions and emissions above 500 mm and low pKa values, i.e. about 6, so they exhibit maximum fluorescence in the physiological pH range of 6-9.

The compounds, covered by the general formula $COUP-(LINK)_n-R$, fall into two general groups.

Group I include those in which n represents zero. In these compounds -R is linked directly to COUP- without the intervening linking group -LINK-. -R is directly released in the presence of an oxidized amine and forms the detectable species.

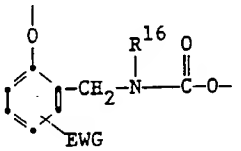
Group II include those compounds in which n equals 1. These compounds are anchimeric releasing couplers. This means that in the presence of an oxidized amine, the -LINK-R portion is released from

COUP- and undergoes an intramolecular reaction to form a heterocyclic ring with concomitant release of -R to form the detectable species.

In both groups of compounds, couplers are known in the photographic arts from which the COUP- component may be easily made. COUP- radicals are disclosed, for example in European Patent Application 0 060 518; U.S. Patent 3,443,940 and U.S. Patent 3,148,062.

The compounds of group I are formed using such known couplers by reacting the latter with compounds containing -R. In general, the reaction is carried out using any of the techniques known in the photographic arts for forming dye releasing couplers. Such methods are exemplified for example, in European Patent Application 0 060 518.

The compounds of group II include the linking group -LINK- between the -R and COUP-. The compounds of this group utilize the same COUP- radicals used in group I. Representative linking groups include:



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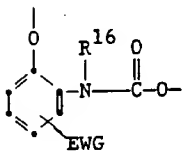

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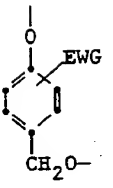

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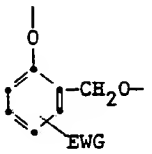


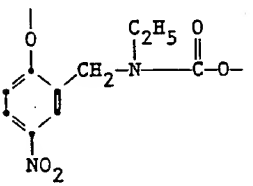
; wherein

R^{16} represents CH_3, C_2H_5, $n-C_3H_7$ or $i-C_3H_7$;

EWG represents an electron withdrawing group in ortho or para position relative to the oxy group -O-, such as $-NO_2$, $-CO_2R^{17}$, $-SO_2R^{17}$, $-SO_2NR_2^{17}$ or -CN,

wherein $-R^{17}$ represents H, alkyl such as methyl, ethyl or octadecyl; or aryl such as phenyl, tolyl or napthyl.

Preferred examples of -LINK- are:



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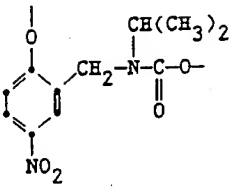

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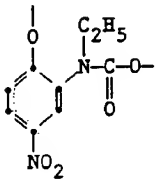

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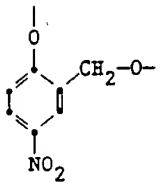
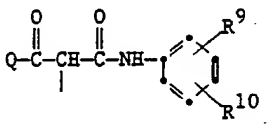


Other substituents can be present in the benzene ring provided they do not adversely affect the rate of coupling or cyclization to release -R.

The anchimeric compounds of group II can be made by conventional methods used to make anchimeric dye releasing couplers of, for example, U.S. Patent 3,443,940 or U.S. Patent 3,148,062.

In general the compounds of groups I and II have structures similar to those disclosed in European Patent Application 0 060 518 and U.S. Patents 3,148,062 and 3,443,940. However, -R, alone or together with ~~-(LINK)-~~, makes the compounds novel.

Examples of the compounds from which COUP- radicals may be formed and which are useful in both group I and II compounds are as follows:



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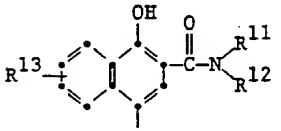

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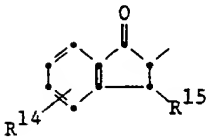


; wherein

Q represents alkyl such as methyl, t-butyl or substituted or unsubstituted aryl such as phenyl, p-methoxyphenyl;

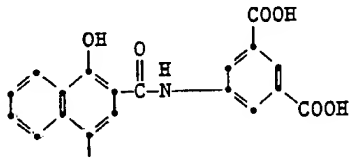
R^9 and R^{10} each independently represent halogen such as chloro or fluoro, hydrogen, nitro, carboxy, sulfo, substituted or unsubstituted carbonamido or sulfonamido; or ureido;

R^{11} and R^{12} each independently represent hydrogen, alkyl such as methyl, ethyl or dodecyl, substituted or unsubstituted aryl such as phenyl or tetradecyloxyphenyl or dicarboxyphenyl;

R^{13} represents hydrogen, halogen, carboxy, sulfo, alkyl such as methyl or ethyl, sulfonamido, carbonamido, etc.;

R^{14} and R^{15} each independently represent hydrogen, halogen such as chloro or fluoro, carboxy, sulfo, alkyl such as methyl, ethyl or hexadecyl, substituted or unsubstituted sulfonamido, carbonamido, etc.

Preferred examples of COUP- include:



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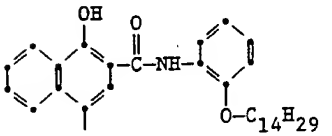

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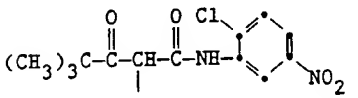

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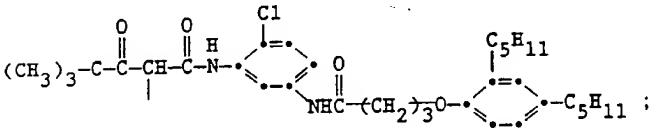

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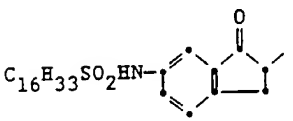

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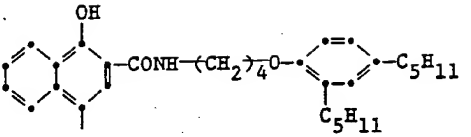

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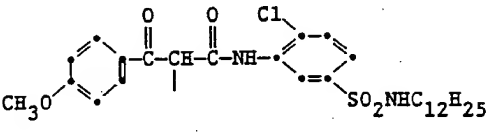
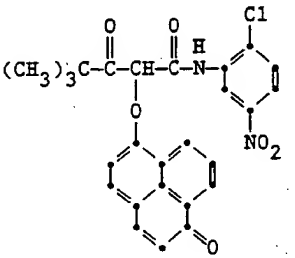
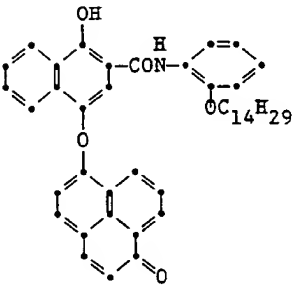
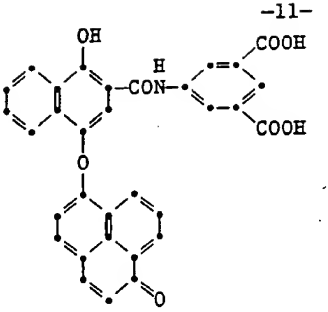


A portion of representative compounds according to group I are presented in Table I.

TABLE I

1)  ;

2)  ;

3) 

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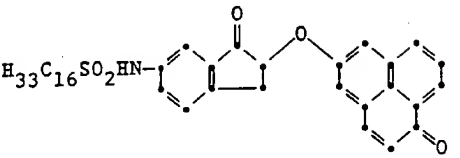
4) 

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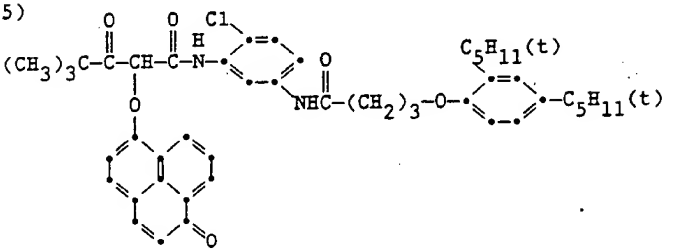
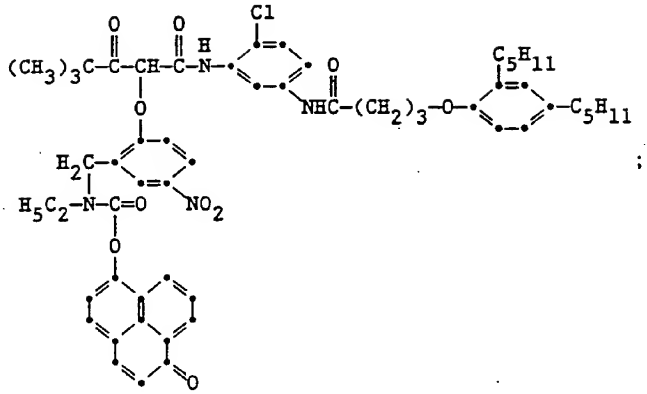
5) 

A portion of the representative anchimeric compounds of group II of the invention are as follows:

TABLE II

1)



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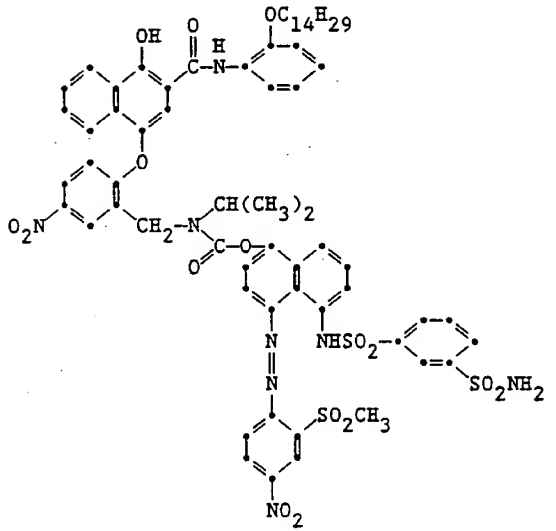

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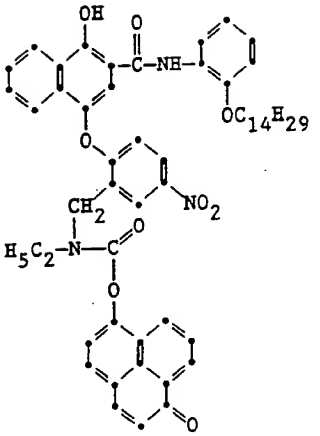

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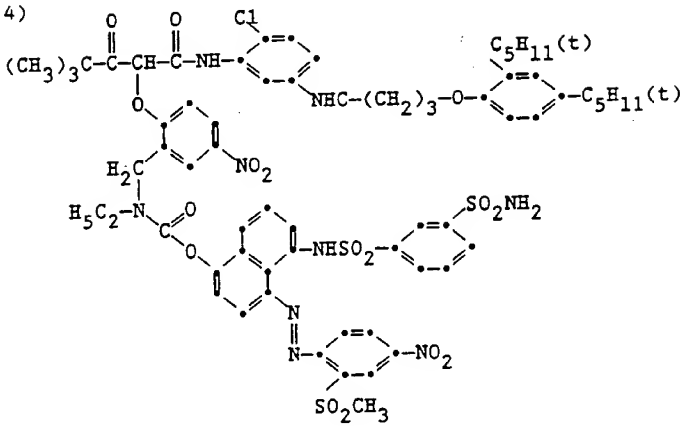
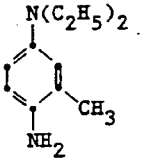


The hydrogen donating primary amines which are useful in this invention are those compounds designated as developers in the photographic arts. Such amines include p-phenylenediamines, p-aminophenols and pyrazolidones. A portion of representative amines are presented in Table III.

TABLE III

1) 

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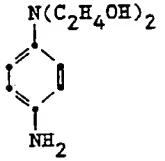
2) 

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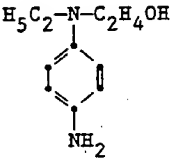
3) 

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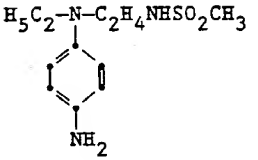
4) 

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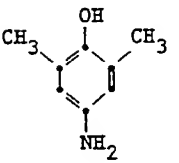
5) 

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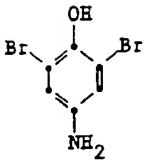
6) 

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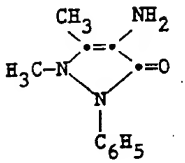
7) 

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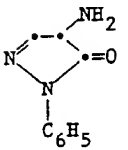
8) 

Detection of oxidase positive organisms is carried out simply by contacting a material, such as an aqueous solution, suspected of containing the organism with a reagent of the invention. The reagent is prepared by dissolving the

COUP—(LINK)$_n$—R compound primary amine in an organic solvent. The relative amounts of each material in the reagent and the choice of solvent are not critical. Anyone skilled in the art will be able to establish the amount of the reagent needed to carry out detection.

The method of this invention can be practiced with a dry analytical element. A variety of different elements, depending on the method of assay, can be prepared in accordance with the present invention. Elements can be configured in a variety

of forms, including elongated tapes of any desired width, sheets, slides or chips. The simplest element can be composed of an absorbent carrier material or water soluble polymer, for example, a thin sheet of a self-supporting absorbent or bibulous material, such as filter paper or strips, which contains the dyes of this invention. A useful element is discussed in commonly owned European Patent Publication 0255087, February 3, 1988. The element comprises a water soluble polymer in which a reagent is included.

The elements can also have two or more discrete zones, either in the same layer or superimposed. At least one of the zones can be a porous spreading zone. The other zones can be reagent zones or registration zones as those zones are known in the art, additional spreading zones, radiation-blocking or filter zones, subbing zones or barrier zones. The zones are generally in fluid contact with each other, meaning that fluids, reagents and reaction products (for example, color dyes) can pass or be transported between superposed regions of adjacent zones. In other words, when the element is contacted with fluid, all reagents of the analytical composition become mixed and can readily move within the element as a composition. Preferably, each zone is a separately coated layer, although two or more zones can be separate areas in a single layer of the element. Besides the references noted above, suitable element components are described also, for example, in U. S. Patents 4,042,335; 4,132,528; and 4,144,306.

Useful absorbent carrier materials are insoluble and maintain their structural integrity when exposed to water or biological fluids such as whole blood or serum. Useful elements can be prepared from paper, porous particulate structures,

porous polymeric films, cellulose, glass fibers, woven and nonwoven fabrics (synthetic and nonsynthetic) and the like. Useful materials and procedures for making such elements are well known in the art as exemplified in U.S. Patents 3,092,465; 3,802,842; 3,915,647; 3,917,453; 3,936,357; 4,248,829; 4,255,384; 4,270,920; and 4,312,834.

The absorbent carrier material can be a porous spreading zone. This zone can be self-supporting (that is, composed of a material rigid enough to maintain its integrity), but preferably it is carried on a separate support. Such a support can be any suitable dimensionally stable, and preferably, nonporous and transparent (that is, radiation transmissive) material which transmits electromagnetic radiation of a wavelength between 200 and 900 nm. A support of choice for a particular element should be compatible with the intended mode of detection (fluorescence, transmission or reflectance spectroscopy). Useful supports can be prepared from paper, metal foils, polystyrene, polyesters, polycarbonates, cellulose esters and others known in the art.

The porous spreading zone can be prepared from any suitable fibrous or non-fibrous material or mixtures of either or both. The void volume and average pore size of this zone can be varied depending upon the use intended.

Useful spreading zones can be prepared using fibrous materials, either mixed with a suitable binder material or woven into a fabric, as described in U. S. Patent 4,292,272, polymeric compositions or particulate materials, for example, beads bound together with or without binding adhesives, as described in U. S. Patents 3,992,158; 4,258,001; and 4,430,436 and Japanese Patent Publication 57(1982)-101760. It is desirable that the spreading

zone be isotropically porous, meaning that the porosity is the same in each direction in the zone as caused by interconnected spaces or pores between particles, fibers or polymeric strands.

The assay method can be manual or automated. In general, in using the dry elements, a determination is made by taking an element from a supply roll, chip packet or other source and physically contacting it with a sample (for example, up to 200 µl) of the liquid to be tested so that the sample and reagents within the element become mixed. Such contact can be accomplished in any suitable manner, for example, by dipping or immersing the element into the sample or, preferably, by spotting the element by hand or machine with a drop of the sample with a suitable dispensing means.

The following examples illustrate the utility of the reagent of the invention in detecting oxidase positive organisms.

Example 1 – Assay for Pseudomonas aeruginosa

This example compares the detection of Pseudomonas aeruginosa (an oxidase-positive organism) and Escherichia coli (an oxidase-negative organism) using dye releasing Compound 1 of Table I which releases a fluorescent dye.

A dispersion of the Compound 1 was prepared as follows. Compound 1 (4 mg) was dissolved in N,N-dimethylformamide (DMF, 250 µL). A surfactant, Triton X-100 (500 µL), was added. The solution was mixed and added slowly with stirring to 25 mL of 0.05 M potassium phosphate buffer (pH 7.5).

Escherichia coli (E. coli) was grown overnight in brain heart infusion (BHI) broth at 37°C without shaking. Pseudomonas aeruginosa (P. aeruginosa) was also grown overnight in BHI broth at

37°C with shaking. About 40 mL of each culture growth was centrifuged, decanted, washed with KP buffer, and suspended in buffer, such that 75 µL of cells in 3 mL buffer gave an OD at 620 nm of 0.448.

The assay was run as follows:

A reagent of the invention was prepared from the Compound 1 dispersion (1 mL), a primary amine, 4-amino-3-methyl-N,N-diethylaniline (25 µL of a 50 mg/mL methanol solution). The reagent was mixed with P. aeruginosa (100 µL) and the buffer, to a final volume of 3 mL.

A control solution was prepared in the same manner, except using E. coli. A background control solution was prepared containing the reagent without the organisms.

Fluorescence was then measured. Excitation was at 540 nm. Emission was at 620 nm. After 7 minutes, the solution containing P. aeruginosa (an oxidase-positive organism) showed a change in relative fluorescence of 52 units, while the E. coli (oxidase-negative) showed a change of only 5 units. The background control showed a change of 12 units, due to aerial oxidation of the developer.

Example 2 – Assay for Pseudomonas aeruginosa

This example compares the detection of P. aeruginosa (oxidase-positive organism) and E. coli (oxidase-negative organism) using the anchimeric dye releasing Compound 1 of Table II which releases a fluorescent dye.

P. aeruginosa and E. coli were grown as described in Example 1. Each were made to a concentration such that 50 µL of cells in a 3 mL cuvette gave an OD at 620 nm of 0.1 unit.

A dispersion of Compound 2 was prepared as described in Example 1 for Compound 1. A test

solution was prepared from the dispersion (1 mL), the primary amine of Example 1 (25 µL), P. aeruginosa (50 µL), and the buffer (1.9 mL). A control solution was prepared as in Example 1 using E. coli. A background control containing all the reagents except organisms was also prepared.

Fluorescence was measured at excitation 540 nm and emission 620 nm. After 10 minutes, the background control and E. coli control showed very little increase in fluorescence. However, the test solution showed a large increase in fluorescence.

Example 3 – Assay for Pseudomonas aeruginosa

This example compares the detection of P. aeruginosa (oxidase–positive organism) and E. coli (oxidase–negative organism) using anchimeric Compound 2, Table II, which releases a cyan dye.

E. coli was grown according to Example 1 and suspended in buffer, such that 100 µL of cells in 3 mL of buffer gave an OD at 620 nm of 0.135. P. aeruginosa was grown according to Example 1 and suspended in buffer such that 75 µL of cells in 3 mL of buffer gave an OD of 0.135.

A dispersion was prepared by dissolving Compound 2, Table II (16 mg) in DMF (1 mL); 250 µL of this solution was mixed with Triton X–100 solution (0.5 mL) and the resulting solution added slowly to 25 mL of 0.05 M of the buffer, pH 7.5.

A test solution was prepared from the Compound 2 dispersion (100 µL), the primary amine (25 µL), P. aeruginosa (75 µL) and the buffer (200 µL).

A control solution was prepared in the same manner, except using E.coli (100 µL). A background control contained all reagents except organisms.

The optical density (OD) was measured at 37°C at 635 nm, and the change in OD was determined after 10 minutes. The OD for P. aeruginosa (oxidase-positive organism) was 1.224. The OD for the E. coli control was 0.211 and the background control was 0.266.

Example 4 - Assay for Pseudomonas aeruginosa with Reagents Coated in a Dry Element

A poly(ethylene terephthalate) support was coated at about pH 6 with a layer comprising surfactant Zonyl FSN, 4-amino-3-methyl-N,N-diethyl-aniline, Compound 3, Table I and poly(acrylamide-co-N-vinylpyrrolidone), 90:10.

A portion of this element (~1 cm^2) was added to a solution containing 3 mL of potassium phosphate buffer, 0.05 M, pH 7.5 and 100 µl of Pseudomonas aeruginosa solution. A second portion of the element was added to a buffer solution without Pseudomonas aeruginosa cells for a background control.

Fluorescence was then measured at excitation 540 nm and emission 620 nm. After 10 minutes, the solution containing the Pseudomonas aeruginosa showed a large increase in fluorescence over the background control.

Claims:

1. A compound having the general formula

$$COUP\text{—}(LINK)_n\text{—}R$$

wherein

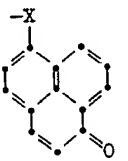
COUP– represents a radical that couples with an oxidized primary amine and releases –LINK–R;

–LINK– represents a divalent radical that undergoes intramolecular cyclization and release of –R upon release by COUP–;

n represents zero or one;

–R represents a monovalent radical that forms a detectable species in the form of a colorimetric dye or fluorescent compound upon release from –LINK–;

wherein –R is selected from the group consisting of:



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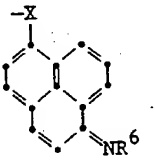

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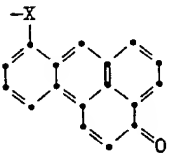
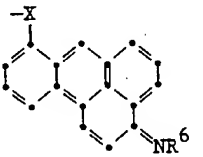


(c) ;



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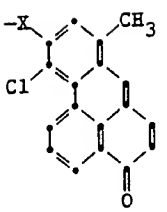

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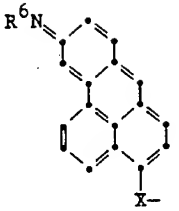

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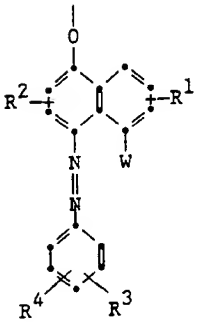

(g)

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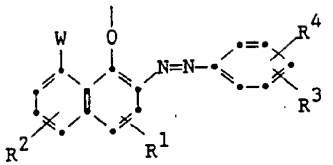
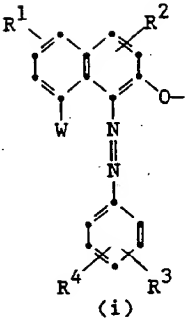


(h)

; and



; wherein

(i)

W represents hydrogen; halogen; hydroxy; substituted or unsubstituted carbonamido; sulfonamido; sulfonyl; ureido or amino;

R^1 and R^2 each independently represent hydrogen, halogen, alkyl, alkoxy, carboxy, sulfo, cyano, nitro, carboxylic acid ester, carbonyl, sulfonyl, carbonamido, sulfonamido, alkylsulfonyl, arylsulfonyl; and

R^3 and R^4 each independently represent halogen, nitro, sulfonamido, sulfonyl, carbonamido, carbonyl, cyano, alkylsulfonyl, arylsulfonyl;

R^6 represents H, CH_3 or C_2H_5;

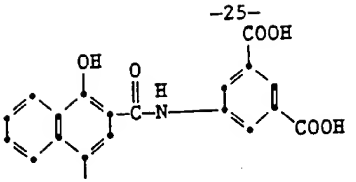
X represents $-O-$, $-S-$ or $-\overset{|}{N}R^5$; and

R^5 represents H, alkyl, cycloalkyl or aryl.

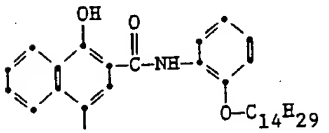
2. The compound of claim 1 wherein

a) the amine is selected from the group consisting of p-phenylenediamines, p-aminophenols and pyrazolidones.

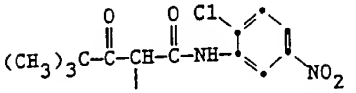
b) COUP- is selected from the group consisting of


OH
O
H
C–N
COOH
COOH

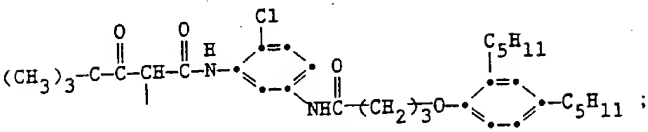
;


OH
O
C–NH–
O–C14H29

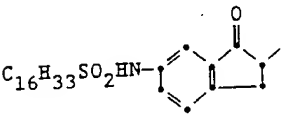
;


O
O
Cl
(CH3)3C–C–CH–C–NH–
NO2

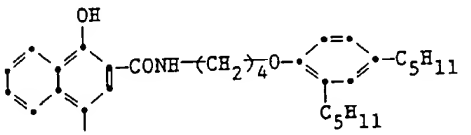
;


O
O
H
Cl
(CH3)3–C–C–CH–C–N–
O
NHC–(CH2)3O–
C5H11
C5H11

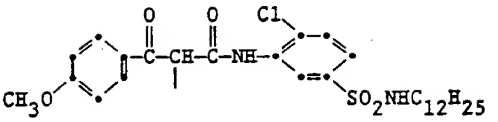
;


O
C16H33SO2HN–

;

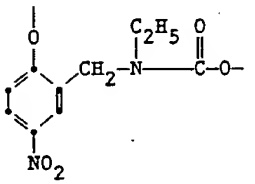

OH
–CONH–(CH2)4O–
C5H11
C5H11

; and



; and

c) -LINK- is selected from the group consisting of



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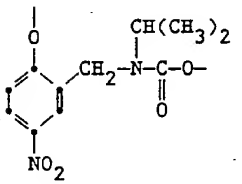

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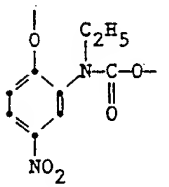

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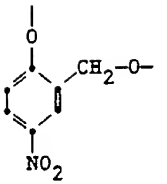
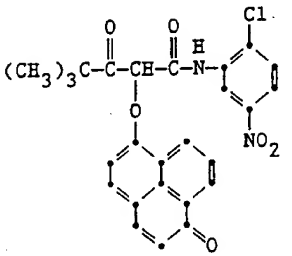


3. The compound according to claim 2 selected from the group consisting of



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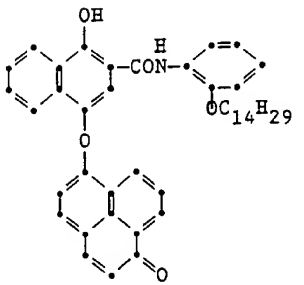

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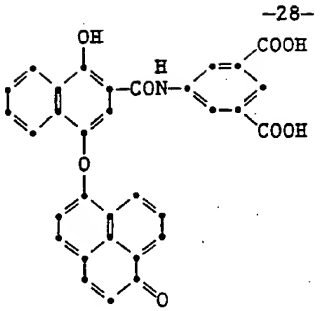

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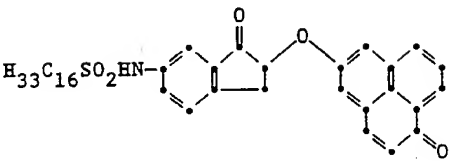

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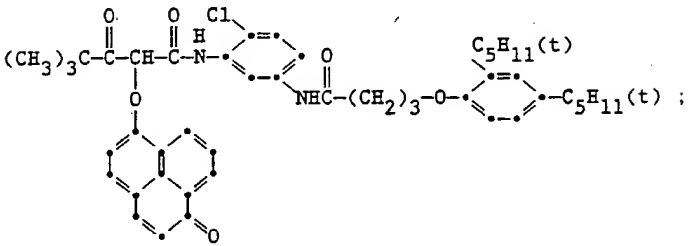

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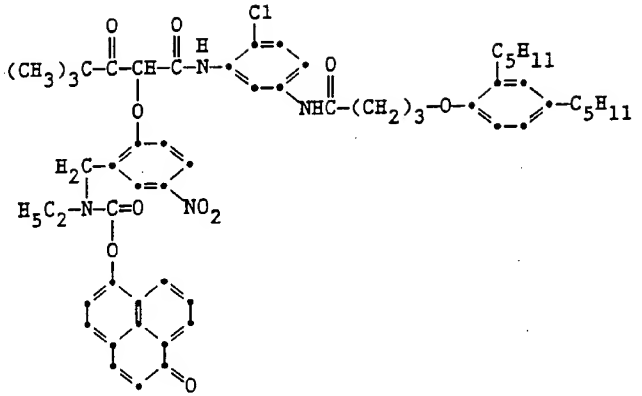

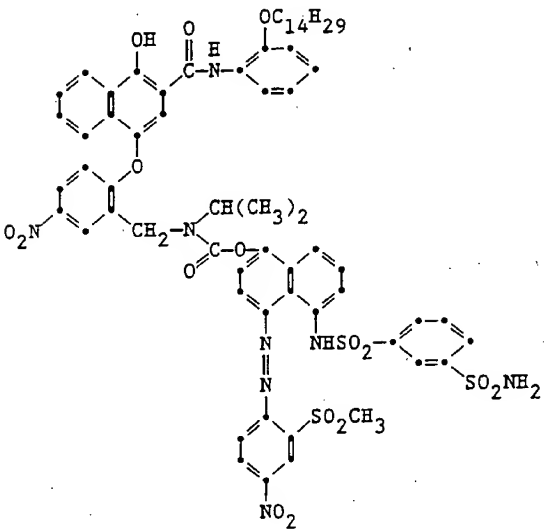

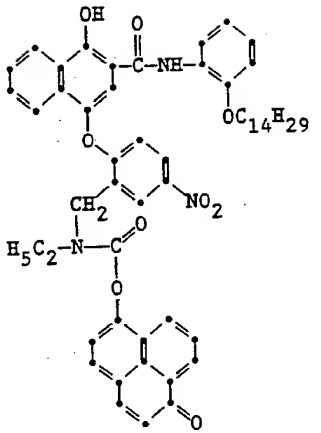

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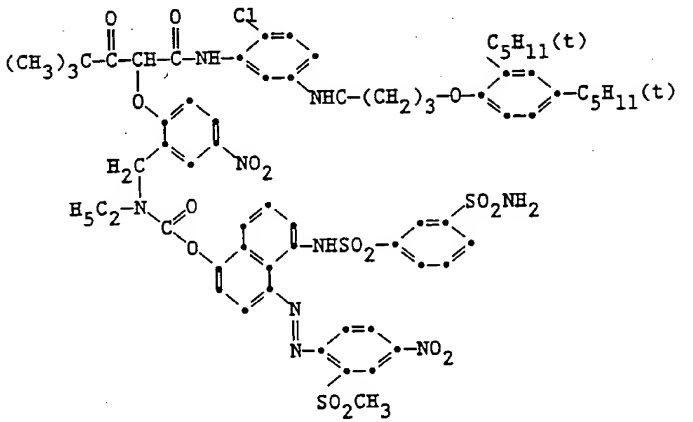

4. A reagent comprising:

a) a hydrogen donating primary amine; and

b) a compound according to claims 1, 2 or 3.

5. A method of detecting oxidase positive organisms comprising the steps of

a) providing a reagent according to claim 4;

b) providing a material capable of containing an oxidase positive organism and

c) mixing an aliquot of a) with the material of b) thereby providing a color or fluorescence in the mixture if the oxidase positive organism is present.

6. An analytical element for detecting oxidase positive organisms in aqueous liquids comprising an absorbent material or a water soluble polymer containing a reagent according to claim 4.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 89/01792

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 12 Q 1/28, G 03 C 7/305, G 01 N 33/52

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	C 12 Q 1/00, G 03 C 7/00, G 01 N 3/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	US, A, 4248962 (P.T.S. LAU) 3 February 1981 see column 10, lines 20-25; column 11, lines 15-19,40-45; column 12, lines 60-68; column 15, lines 1-30 --	1,2,3,4
P,X	EP, A, 0296794 (EASTMAN KODAK CO.) 28 December 1988 see page 3, lines 15-21; claim 5 --	1,2,3,4
X	EP, A, 0173302 (FUJI PHOTO FILM CO. LTD) 5 March 1986 see pages 54-58 --	1,2,3,4
A	EP, A, 0060518 (FUJI PHOTO FILM CO. LTD) 22 September 1982 see page 30, lines 1-11; page 31, lines 6-11 cited in the application --	1-5

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
3rd August 1989	20. 09. 89
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	T.K. WILLIS

International Application No. PCT/US 89/01792

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	EP, A, 0231125 (EASTMAN KODAK CO.) 5 August 1987 see pages 5-6 --	1,5,6
A	EP, A, 0231126 (EASTMAN KODAK CO.) 5 August 1987 see claims 1-5 --	1,5,6
A	EP, A, 0232130 (EASTMAN KODAK CO.) 12 August 1987 see claims --	1,5,6
A	DE, A, 1955901 (AGFA-GEVAERT AG) 13 May 1971 see page 9, lines 28-32; page 10, lines 1-19 ----------	1,4,5

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 8901792 SA 28595

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on 08/09/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 4248962	03-02-81	BE-A- 873046	22-06-79
		CA-A- 1134818	02-11-82
		DE-A,C 2855697	28-06-79
		FR-A,B 2412872	20-07-79
		GB-A,B 2010818	04-07-79
		JP-A- 54145135	13-11-79
EP-A- 0296794	28-12-88	US-A- 4774181	27-09-88
		JP-A- 1021446	24-01-89
EP-A- 0173302	05-03-86	JP-A- 61184541	18-08-86
		US-A- 4711837	08-12-87
EP-A- 0060518	22-09-82	JP-A- 57150399	17-09-82
EP-A- 0231125	05-08-87	US-A- 4812409	14-03-89
		JP-A- 62215399	22-09-87
EP-A- 0231126	05-08-87	US-A- 4812393	14-03-89
		JP-A- 62223147	01-10-87
EP-A- 0232130	12-08-87	US-A- 4803161	07-02-89
		JP-A- 62190142	20-08-87
DE-A- 1955901	13-05-71	BE-A- 758415	03-05-71
		FR-A- 2082968	10-12-71
		GB-A- 1332692	03-10-73
		US-A- 3694207	26-09-72

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